# Webinar Q&A Report:
# From Pregnancy to Menopause: Studies of Physical Activity, Behavior, and Energy Balance in Mice

1. **How soon in early pregnancy does the timing of food consumption change (ie. to eat more around-the-clock rather than primarily during the dark period) and is this connected to the change in running wheel activity?**

   S. Ladyman: We see food intake start to significant increase from about mid pregnancy onwards in our mice. In our experiments they don't really change the percentage of their total food intake they eat in each phase of the light cycle, so pre-pregnancy they are eating about 25% of their food in the light phase and this is about 30% by late pregnancy (days 16-18) but this difference is not significant. I don't think we have any evidence that a change in when they are eating their food is connected to the change in running wheel activity. However, increased body weight and physical constraints due to increased size as gestation advances are highly likely to greatly contribute to the reductions in running wheel activity in the second half of pregnancy. The initial change in running wheel activity during early pregnancy is not associated with any change in body weight or size. Alongside the role of increased body size in reducing running wheel activity, our data from mice with the forebrain specific deletion of prolactin receptors would indicate that even in late pregnancy prolactin may contribute to the suppression of running wheel activity, as these KO mice run more in late pregnancy and their body weights are not different to controls.

2. **In the trace of running wheel activity in an individual mouse across pregnancy, lactation, and post-weaning, was the immediate post-partum peak frequently observed in other mice? What do you think is happening here?**

   S. Ladyman: Yes, it was frequently observed. Mice ovulate soon after giving birth and this is called the postpartum ovulation. From what we know about this in rodents it is likely associated with a rise in estrogen soon after birth similar to increased estrogen prior to the LH surge in cycling female mice, and we predict that this estrogen surge drives this increase in running wheel activity for this one night.

3. **Regarding your findings of the importance of MPOA-specific expression of prolactin receptor: Are there changes in prolactin receptor in these neurons with aging or prior breeding? Older dams and inexperienced dams seem to abandon litters more frequently—perhaps these pathways are disrupted in these cases?**

   S. Ladyman: In rats there is some evidence to suggest that prolactin receptor express increases with

reproductive experience, but I don't think it has been looked at in mice and we have some studies planned to address this question in the future. It is highly likely that impaired prolactin action in the MPOA may be one of the causes of litter loss. Currently, there is not too much work being done on understanding why some mice abandon their pups after birth and some don't (although I will point you in the direction of PMID: 21338647 and DIO 10.1111/j.1439-0531.2012.02147.x for some related reviews). We do know that prolactin receptor expression does not change from virgin to lactating mice in the MPOA.

4. **Do you think nutritional supplementation to enhance metabolic pathways that rely on Pts-produced BH4 could promote metabolic health in post-menopausal women?**

   V. Vieira-Potter: We know that aging and estrogen loss absolutely affect brain health in many ways, and those catecholamines that are produced from BH4 are critical for many neurological processes (e.g., learning, memory, circadian rhythm, etc.). We are now understanding that brain changes also directly affect systemic metabolism. My lab is very interesting in exploring the efficacy of dopamine activators to improve brain health and metabolism. Whether there are nutritional supplements that may improve metabolism by acting on these pathways - I do not know, but this would be an important area to explore.

5. **Have you investigated the lymphatics at all in the context how estrogen may play a metabolic protective role?**

   V. Vieira-Potter: I have not directly assessed the lymphatic system in settings of estrogen loss, but we have assessed immune cell populations in adipose tissue following estrogen loss in rodents and found that estrogen loss does affect those immune cells - that is, loss of estrogen increases inflammatory cell populations. Estrogen may also activate M2 macrophage phenotype switching, favoring a less inflammatory response.

6. **When do the metabolic changes start to occur in ovariectomized mice?**

   V. Vieira-Potter: We see weight gain and insulin resistance develop at least by 12 weeks following ovariectomy. The weight gain occurs quite early - within the first couple of weeks following surgery.

7. **What would happen if you conduct these experiments under thermoneutrality? Would you also see these easy changes in PRL and running wheel activity?**

   S. Ladyman: This is a great question and something we are planning to do in the future. My hypothesis is that during pregnancy mice would run even less at thermoneutrality or more accurately, they would change their running wheel activity levels by a greater degree than the change at 22C that we saw in our current data. If prolactin is involved in increasing sensitivity to heat during pregnancy and more quickly activating behavioural and/or physiological changes to prevent raising body temperature, then at a warmer ambient temperature we would expect bouts of running wheel activity to stop earlier to prevent increases in body temperature. This is very speculative though! We have some data showing thermoregulating pathways in the POA are prolactin sensitive but we have a long way before proving or disproving this idea that changes in thermoregulation underlie this change in running wheel behaviour.

8. **You mentioned that moms who abandon pups spontaneously run more during late pregnancy. Do you think this is an inherent issue on the mom's side, or do you think activity relates to altered pup development, which may lead moms to abandon pups?**

   S. Ladyman: I don't think we can make any strong conclusions about this from this data. I think that this increase in running wheel activity in late pregnancy in mothers who go on to abandon their litter is just an indicator that something is not completely right with these pregnancies, and that this pregnancy outcome is not solely determine on something that happens at parturition, like a stressful birth. The increase in running wheel behaviour may just be a reflection of the fact that these mice which go on to abandon their pups tend to have a lower weight gain during pregnancy, which in itself could reflect an issue with pup development and growth. It might also indicate that there is an issue (reduction) with prolactin/placental lactogen levels and that might mean maternal/nursing behaviour and/or milk production is impaired. There are many issues that may lead to litter abandonment and likely it is not one thing that accounts for all cases. What makes it hard to study is that everything is retrospective, as we don't know who will abandon pups until they do it and a large starting group size is needed to get a useful number of mice that abandon their pups to be able to look for correlations.

9. **Do you think, that if we correct the sleep habits in menopausal women, they may not develop weight gain?**

   V. Vieira-Potter: I don't know of studies that have looked at this, but we do know that insomnia and sleep disturbances occur in postmenopausal women and coincide with metabolic dysfunction. We also know that sleep loss causes insulin resistance and weight gain - so a very cool study would be to test whether improving sleep among postmenopausal women is sufficient to improve their metabolism and lessen weight gain! Lovely idea!! We are actually performing studies currently in humans where we are assessing the effects of sleep restriction on adipose tissue metabolism. Look out for our studies in the upcoming years!

10. **Have you managed to measure activity of another enzymes involving dopamine and phenylephrine synthesis?**

    V. Vieira-Potter: We have not! We were so excited about the Pts finding and because there is evidence that estrogen affects dopamine metabolism, we are anxious to assess the efficacy or dopamine-acting drugs on metabolism and physical activity behaviors in women following menopause. We're hoping to start some clinical work in the next few years, so stay tuned!

11. **Do we see similar results in all the discussed parameters in women diagnosed with PCOD/PCOS and the normal population?**

    V. Vieira-Potter: Such a great question! There are interesting parallels between PCOS in young women and menopause in older women. In both conditions, obesity an insulin resistance develops and the ratio of estrogen:testosterone changes. Physical inactivity also tends to coincide with both conditions as well - very interesting indeed!

**12. Do you see the same changes in spontaneous activity?**

S. Ladyman: When there is no running wheel present, in early pregnancy mice do show a rapid reduction in spontaneous, home cage activity. When a running wheel is present, in early pregnancy we don't see any changes in spontaneous, home cage activity, and in fact as pregnancy advances, we see more home cage activity compared to the virgin state. This latter effect might be because mice are spending less time on the running wheel and hence moving around the cage more. With our forebrain specific Prlr KO mice and the GABA specific Prlr KO mice, we don't see any change in home cage spontaneous activity compared to WT during pregnancy, but this is with the wheel present, when we do see changes in wheel running. We have yet to repeat the experiments to look at home cage spontaneous activity in these transgenic mice in the absence of running wheels, which would give us a clearer picture of what is happening to any effect of prolactin action in the brain on home cage activity during pregnancy.

**13. Do you see the same response in multiparous dams compared to first-time dams?**

S. Ladyman: We have not yet looked into this but are planning to do this experiment soon. Work from reproductively experienced rats suggests that they become more sensitive to prolactin so we might hypothesize that in subsequent pregnancy this effect might be more pronounced. Equally, it might not change, as prolactin secretion might be lower in subsequent pregnancies (as it is in the non-pregnant state in reproductively experienced rats but currently, we have little data on this in reproductively experienced mice and none in subsequent pregnancies) so with lower prolactin but increased sensitivity the effect might work out to be the same. Obviously, lots more work to do here.

**14. What accounts for the spike in running just after birth?**

S. Ladyman: Yes, it was frequently observed. Mice ovulate soon after giving birth and this is called the postpartum ovulation. From what we know about this in rodents it is likely associated with a rise in estrogen soon after birth similar to increased estrogen prior to the LH surge in cycling female mice, and we predict that this estrogen surge drives this increase in running wheel activity for this one night.

**15. Which factors most explained the reduction in running behavior? For example, did mice run more slowly or did the mice just spend less time running - or a little of both?**

S. Ladyman: We have shown that running speed does not significantly decrease until late pregnancy (days 16-18), although it does begin to drop a bit earlier (days 13-15) this time point didn't reach statistical significance. Therefore, at least the initial early pregnancy drop is due to reduced amount of time running. I have not yet analyzed the speeds of our various Prlr KO mice so I should probably get that done, but my guess would be that running speed during early pregnancy is the same as the virgin state. What I am interested in assessing is whether in early pregnancy the mice are engaging in less bouts of running or if each running bout is of a smaller duration. I think this might give some insight into underlying mechanisms.

16. **What did your sham for OVX entail?**

    V. Vieira-Potter: We make an incision, expose the ovary, replace the intact ovary, and suture.

17. **Menopause-related weight gain may also be due to age-related physical inactivity, is that also evident in males?**

    V. Vieira-Potter: Yes, physical activity levels reduce with aging to some degree in both sexes. This likely contributes to weight gain in both sexes.

18. **In DIO mice, is the locomotion disrupted by the obese state? If yes, can these hormonal alterations influence neuronal populations that control locomotor activity?**

    V. Vieira-Potter: We have actually seen that locomotor activity is reduced with high fat diet feeding and obesity in rodents. As far as the brain-specific mechanism, we do not know, but this is an important area of research! Maybe dopamine is involved!

19. **Do progesterone levels affect physical activity?**

    S. Ladyman: I think there is a long-standing assumption that progesterone suppresses physical activity including running wheel activity but there is not a great amount of data to support this. This review gives a bit more insight: Int J Biol Sci 2008; 4(3):126-132. doi:10.7150/ijbs.4.126. If not for our prlr KO studies I would have just assumed that increased progesterone during early pregnancy drives this early reduction in running wheel activity, but as far as I can find in the literature progesterone doesn't increase for a few days following mating so it is unlikely to be driving the reductions on days 1 and 2 of pregnancy. We are about to measure progesterone in our forebrain specific Prlr KO mice on day 3 of pregnancy, just to make sure they are the same as controls. In fact, it is possible they will be elevated due to the higher prolactin levels in these mice due to prolactin's stimulation of progesterone in the ovary.

20. **Are the high levels of prolactin during lactation contributing to the very low levels of running wheel activity in lactation?**

    S. Ladyman: Our forebrain specific Prlr KO mice do run more than WT for about the first week or so of lactation suggesting a role for prolactin here too. For the rest of lactation (about another two weeks) what data we have, indicates no difference between WT and KOs so there are definitely other factors contributing at this stage.

21. **How does prolactin reach the brain? Could this be mediated by neuronal prolactin?**

    S. Ladyman: Prolactin is transported across the blood brain barrier by an active transport system, and we really don't know very much about the details of this process. We have shown that there is decreased prolactin transport into the brain during mid-late pregnancy, but it is likely that this is competing with the high endogenous placental lactogen at this time making it a little hard to interpret this result. In lactation, prolactin transport into the brain is increased. We assume that prolactin and/or its homologue, placental

lactogen, are increased in the brain as well as in blood during these states, and our work (and others) showing higher basal levels of pSTAT5 in various brain regions in pregnancy and lactation support this assumption. There is some evidence that prolactin is produced in the brain as well as the anterior pituitary, and the MPOA is one site that prolactin mRNA has been detected so it is possible that neuronal prolactin contributes to this suppression of running wheel activity during pregnancy, however we have not specifically investigated this.

**22. Is this effect of prolactin specific to females? What happens in males?**

S. Ladyman: The effect of acute prolactin to reduce running wheel activity is specific to females as when we repeated this experiment in males, we saw no effect of prolactin.

23. **Was there any difficulty in the Prolactin receptors knockouts getting pregnant?**

S. Ladyman: Our forebrain specific Prlr KO mice do have some extra challenges to getting pregnant. Prolactin's secretion from lactotrophs in the pituitary is regulated via an inhibitory process. Tuberoinfundibular dopamine neurons in the arcuate nucleus release dopamine to inhibit prolactin secretion, and prolactin acts back on these TIDA neurons to promote dopamine release, thus creating a negative feedback loop to maintain prolactin secretion. In our forebrain specific Prlr KO mice this feedback loop is disrupted due to the lack of Prlr on TIDA neurons, thus these mice have high levels of prolactin, which can act on the ovary to impair the estrous cycle. These mice have extended estrous cycles and only are in proestrous (day of the estrous cycle when female mice will mate) about once every 12-14 days instead of every 4-5 days like a WT mouse. So, it can take longer to get them pregnant, but they do get pregnant. We haven't seen this in any of our other area/neuron population specific Prlr KO mice, where Prlr have not been removed from the TIDA neurons.

**24. Did you appreciate regionality differences in the visceral adipose depot when measuring UCP1 expression levels?**

V. Vieira-Potter: We only look at a small section of tissue, so it is difficult to say. We do see "clusters" of adipocyte "beiging" in some studies and it is not clear what explains this regionality.

**25. Can you comment on the potential role of increased FSH in postmenopause and its role on these observations? For example, premenopause females who have disruption to the HPA axis (Anorexia nervosa and RED-S are examples); I believe have low E2, but not the same symptomology (but I believe these women also have low FSH - a primary difference).**

V. Vieira-Potter: Interesting question - there was one study in rodents which showed that FSH levels suppressed UCP1, actually. We recently published a paper in humans (Porter et al Obesity 2020) where we found a positive relationship between an inflammatory marker in the blood and FSH levels. Interestingly, we actually found an inverse correlation between FSH and WAT UCP1. Interesting about HPA axis disruption in both settings of low E2.

If you have additional questions for Sable Systems International regarding content from their webinar or wish to receive additional information about their products and laboratory services, please contact them by phone or email:

## North America

Sable Systems International
Headquarters and North America Sales
3840 N. Commerce Street
North Las Vegas, NV 89032 U.S.A.

Tel: 1-800-330-0465 / +1 (702) 269-4445 (U.S.)
Tel: +1-866-217-6760 / +1 (702) 269-4445 (Canada)

Email: sales@sablesys.com

## Europe

Sable Systems Europe GmbH
Ostendstr. 25
D-12459 Berlin, Germany

Tel: +49-30-5304-1002
Mobile: +49-176-2078-7008
Fax: +49-30-5304-1003

Email: sales@sablesys.com